

Effects of Heavy Metal Contamination on Soil Microbial Activity

An Honors Thesis (HONRS 499)

by

Jennifer K. Kooy

Thesis Advisor  
Dr. David LeBlanc

David C. LeBlanc

Ball State University  
Muncie, Indiana

April, 1996

Graduation: May 4, 1996

SpCol  
Thesis  
2.4  
1996  
SpCol

## ABSTRACT

This project involved assessing the effects of heavy-metal contamination on soil microbial community activity. A literature review discusses the effects of varying concentrations of heavy metal contamination on the activity of the soil microbial community, as measured by the health and vigor of soil. This is followed by a case study research project which elucidates the effects of heavy metals on the soil microbial community at J-Field, Aberdeen Proving Ground, Maryland. This thesis project is an extension of my experience as a Summer 1995 Student Research Participant at Argonne National Laboratory, located in Argonne, Illinois.

## INTRODUCTION

This thesis project is based on an internship in which I participated during the summer of 1995. As a Student Research Participant, I worked for the Environmental Assessment Division (EAD) of Argonne National Laboratory, located in Argonne, Illinois. The EAD was involved in an ecological risk assessment (ERA) at eight sites of J-Field in the Edgewood Area of Aberdeen Proving Ground in Harford County, Maryland (Figure 1). This area has been used since World War II to develop, manufacture, test, and destroy chemical agents and munitions. The ERA is part of an even larger ongoing project, consisting of a remedial investigation/feasibility study of the area. The ERA is broken up into three phases. Phase 1 looks at assessing the ecological effects of contamination, including biotic inventories, residue analyses, selection of contaminants of ecological concern, acute and chronic toxicity tests, and preliminary identification of receptors and endpoints. Phase 2 includes definitive toxicity testing to determine how organisms are affected by contaminated environmental media, additional residue analyses, and development of pathway models for estimating dose to higher trophic level receptors. Phase 3 involves pathway analyses to estimate contaminant dose to receptors and development of site-specific benchmark values for use in estimating risk (Martino et al., 1995).

My project was a Phase 1 study concerning only one of the eight sites of J-Field: the toxic burning pits (TBP) (Figure 1). The pits were used between the late 1940s and the 1960s to dispose of such items as chemical agents, bulk chemical wastes, drummed chemical wastes, high explosives (by open burning and open detonation), nerve agents, incapacitating agents (also known as riot control agents), chlorinated solvents, and blister agents (Martino et al., 1995). As a result, the soil in this area has been contaminated with many heavy metals. Work has already been done to determine the levels of contamination

in the TBP area. My project focused on relating the level of soil microbial activity to the level of heavy metal contamination.

The ultimate goal of the ERA is to be able to perform a risk evaluation in order to characterize the risk to ecological resources from the current levels of contamination at J-Field. The risk evaluation will be conducted for J-Field as a whole and for each of the eight sites individually. Ecological risk will be evaluated by estimating an ecological effects quotient for each contaminant of concern and each ecological receptor. Risk will also be evaluated using a weight-of-evidence approach, which considers the results of all the laboratory and field studies. The risk evaluation will discuss the ecological significance of any observed or predicted risks (Martino et al., 1995).

I have included a literature review of soil microbial activity as it relates to heavy metal contamination, including different methodologies used for this purpose. I have also included a case study utilizing one particular method for determining soil microbial activity applied to the TBP site of J-Field.

## LITERATURE REVIEW

The soil microbial community is an essential component of terrestrial ecosystems. Microbial communities are the main acting agents for most soil biogeochemical processes and have the ability to interact with the primary productivity of ecosystems by regulating nutrient availability and degradation pathways of soil contaminants (Jenkinson and Ladd, 1981; Okano et al., 1989; Singh et al., 1989). The fertility of the soil ecosystem depends on the activity of soil microorganisms as they mediate the turnover of the soil organic matter (Brookes, 1995). Contamination of the soil may alter soil processes, including immobilization and mineralization of nutrients controlled by these microorganisms.

Microorganisms are the ideal indicators of soil pollution because both their mass and activity are closely related to the soil microenvironment (Brookes, 1995). Often microbial biomass and biomass activity measurements are used to determine the effects of contamination on the soil community. Contamination affects many soil processes which are important for maintaining normal nutrient cycling in all ecosystems (Coleman, 1985; Dindal, 1990; Ingham et al., 1986a,b). Plant growth is dependent on the microbial immobilization and soil foodweb interactions to mineralize nutrients. In undisturbed ecosystems, the processes of immobilization and mineralization are tightly coupled to plant growth, but following disturbance, this coupling may be lost or reduced. Nutrients may be no longer retained within the system, causing problems for systems into which nutrients move (Ingham and Coleman, 1984; Hendrix et al., 1986; Nannipieri et al., 1990). Measurement of disrupted processes may allow determination of a problem long before normal cycling processes are altered (Ingham et al., 1986a,b), the natural vegetation is lost, or human health problems occur. By monitoring soil microorganism dynamics, we can detect detrimental ecosystem changes and possibly prevent further degradation (Ingham and Coleman, 1984).

Soil microbial activity is often disturbed by metal contamination. Metal pollution can occur in many forms, the principal ones being: mining, metallurgical and industrial wastes, automobile exhausts, and land disposal of sewage sludges (Dumontet and Mathur, 1989; Brookes, 1995). The most common heavy metals include Cu, Ni, Cd, Zn, Cr, and Pb (Brookes, 1995). Németh et al. (1993) reported that large amounts of heavy metals such as Cd, Cr, Ni, Pb, and Zn are often present in sewage sludges and waste waters. Several heavy metals, such as Cu, Zn, and Fe, are essential for the normal growth of microorganisms, but may become toxic at high concentrations (Dick, 1991). Some heavy metals are more toxic than others: Bååth (1992) found that the toxicity of different metals to bacteria decreased in the order  $\text{Ag} > \text{Cu} > \text{Cd} > \text{Zn} > \text{Pb}$ .

Dick (1991) states that heavy metals are toxic for three reasons. First, heavy metals block the essential function of biomolecules. Second, a heavy metal ion may displace other essential metal ions in biomolecules. Lastly, heavy metals can modify the active conformation of biomolecules. Heavy metal pollution would also be expected to have detrimental effects on the soil microbial community because the metals would inhibit the enzymatic activities of the microorganisms (Tyler, 1974, 1975, 1976; Jordan and Lechevalier, 1975; Freedman and Hutchinson, 1980; Mathur, 1981). However, there has not been much evidence collected that conclusively supports this effect on the microbial community. Ortiz and Alcañiz (1994) found soil respiration activity to increase along with increasing sewage sludge concentration. Likewise, Bardgett and Saggiar (1994) found that respiration was greater in heavily contaminated soil than in less contaminated soil. However, Yeates et al. (1994) found that biological activity was depressed at high levels of Cu, Cr, and As contamination. McGrath (1994) states that soil respiration may not be as influenced by low levels of metal contamination as microbial biomass; respiration rates seem to be depressed only at high metal concentrations. In accordance with this idea, Starzecka and Bednarz (1993) found a decrease in bacterial biomass when metallurgic

dusts were present. Any effect on the microbial community from heavy metals would be expected to be permanent, due to their toxicity and persistence (Brookes, 1995).

Other effects of heavy metals on the microbial community can include reduction of microbial diversity and selection for strains resistant to the toxicity of heavy metals (Dick, 1991). Angle et al. (1993) found that there are minimal bacterial population changes in soils heavily contaminated with metals, as most soil bacteria are resistant to high metal concentrations. According to Dick (1991), there are many mechanisms by which microorganisms can become tolerant or resistant to heavy metals. The plasma membrane of the organism may not be permeable to heavy metals, therefore limiting uptake. Some microorganisms accumulate the metal in a cellular location or in a form that will not hinder the biological processes which are sensitive to the effects of the metal. The toxic effects of the metal may also be reduced by the microorganism by making the metal less soluble or by removing the metal from the environment by forming volatile metabolites.

There are many different mechanisms organisms can use to overcome the effects of metal contamination, including: avoidance, exclusion, immobilization, excretion, and those involving enzymatic change (Tyler et al., 1989). As just indicated, heavy-metal tolerant fungi and bacteria often use an immobilization mechanism as their main defense against high concentrations of metals: the organisms bind the metals to the cell wall in order to immobilize them (Tyler et al., 1989). Microorganisms have the ability to interact in a variety of specialized ways with metals. For example, some microorganisms are able to accumulate and immobilize trace metals and are even capable of crystallizing them (Lepp, 1992). Bacteria are able to produce extracellular polymers that can form capsules or loose aggregates around cells; their anionic properties then allow them to bind to metal cations (Lepp, 1992). Fungi are also capable of accumulating metals (Lepp, 1992).

Although many organisms are heavy-metal tolerant, they may lose some of their ecological productivity as a result (Tyler et al., 1989). This may mean that they are more susceptible to natural environmental change.

Not all microorganisms are partially or totally resistant to the effects of heavy metal pollution. Aoyama et al. (1993) found that Cu accumulated in soils decreased the soil microbial biomass considerably. Inefficient biomass synthesis may be the cause of reduced biomass in heavy metal contaminated soils (Chander and Brookes, 1991b). Heavy metals might also change the composition of the microbial population by suppressing or killing sensitive parts of the microbial community (Fließbach et al., 1994). Rühling and Tyler (1973) found that Cu, Ni, Zn, Cd, and Pb affected the biological activity of forest soils negatively. Generally, a disturbance will result in lower taxonomic and genetic diversities of the organisms (Barreiro and Pratt, 1992; Reber, 1992). Physiological versatility was found to be a characteristic of organisms that were able to adapt to a disturbed community (Barreiro and Pratt, 1992).

A common way to assess the effects of pollution in soil is by using soil microbial biomass measurements. How healthy the soil microbial population is in its environment is often related to the amount of soil microbial biomass present in the soil (Jenkinson, 1987) and its level of activity. For this reason it is important that a fairly accurate assessment of the microbial biomass in the soil can be made so that the health of the microbial population might be qualified. A number of factors affect biomass estimations, including the number and types of microorganisms present, incubation temperature, soil type and preparation, and storage of soil samples (Hendricks and Pascoe, 1988). No method to determine microbial biomass has been found to be suitable for all soil types because each soil is dependent on factors such as water, temperature, and nutrient status that are constantly fluctuating (Hulm et al., 1991).

Hulm et al. (1991) describes two general methods that allow these problems to be overcome. One method involves the pre-incubation of soil under standard conditions followed by the stimulation of the microbial community by the addition of a substrate, such as glucose. The other method involves killing the biomass in order to determine the total population. In this method, total population can be determined either by the



mineralization of the killed biomass or by extraction of the cellular components of lysed microbes (usually extraction of biomass C or biomass N). However, no one method will give an unequivocal biomass measure. Hulm et al. (1991) also describes several limitations to determining soil microbial biomass that these two general methods present. One limitation is that biomass stimulated by a substrate may not have had its entire microbial population stimulated. With killed biomass, there is no way to differentiate between dead or living (inactive or active) cells. Another problem with using a killed biomass technique is that there is an assumption that the microbial population was in a constant physiological state, when in fact any number of stresses could have led to a fluctuation in the components of the biomass, leading to anomalous results. Any number of factors can affect a measurement of biomass and result in faulty data.

There are many specific procedures within the two broad methodological categories just described as well as other techniques that are available to measure microbial biomass. Each procedure has its advantages and disadvantages. These methods include: chloroform fumigation methods, substrate-induced respiration (SIR), direct microscopy, ATP concentration, ninhydrin-reactive nitrogen (NRN) analysis, dehydrogenase activity, and fluorescein diacetate (FDA) hydrolysis.

Chloroform fumigation methods are widely used measures of soil microbial biomass. There are two basic chloroform fumigation methods: fumigation-incubation (FI) and fumigation-extraction (FE). Both methods involve the initial step of fumigating the soil samples with chloroform. The FI method then follows with an incubation period, after which the amount of CO<sub>2</sub> released is measured and related to the biomass. Bacteria, actinomycetes, microfungi, algae, and microfauna are included in this method (Badia and Alcañiz, 1993). One major limitation of this method is obtaining a proper unfumigated control sample (Smith and Paul, 1990; Wardle and Ghani, 1995). Another disadvantage is the length of time that is involved in order to assess the soil biomass (Smith et al., 1985).

This method may be limited by soil acidity but may not be limited by the presence of heavy or transition metals (Dumontet and Mathur, 1989).

Unlike FI, the FE method follows the chloroform fumigation with extraction of biomass C (or N), usually using  $K_2SO_4$ . This method has the ability to measure both biomass C and biomass N. It has been found to be useful for a wide range of soils, both natural and disturbed, as well as being resistant to handling errors (Joergensen and Brookes, 1991). It can also be used shortly after the addition of substrate (Ocio and Brookes, 1990) and in the presence of plant roots (Mueller et al., 1992). Both fumigation methods have the possibility of having problems with the efficiency of fumigation (Ingham and Horton, 1987), though most studies have not found problems with chloroform as a fumigant (Jenkinson and Powlson, 1976; Wardle and Parkinson, 1990; Sankrukova, 1992).

Substrate-induced respiration (SIR) is another common method used for determining soil microbial biomass and activity. This method involves the addition of a substrate (usually glucose) to a soil sample in order to induce a maximum respiration rate from the soil microbial biomass (Smith et al., 1985). The  $CO_2$  production rate is then measured and can be converted to biomass C values. This method provides a measure of total active microbial biomass, in contrast to the chloroform fumigation methods which measure total microbial biomass (Wardle and Ghani, 1995). Harden et al. (1993) found that SIR may not be applicable to pesticide-contaminated soils. Their reasons include the fact that the calibration factor used to convert SIR to microbial biomass was not established on pesticide-contaminated soils and that the pesticide contamination might alter the proportion of organisms able to mineralize glucose. However, Hulm et al. (1991) found that the SIR method was the most sensitive of those methods tested, and had the advantages of being adaptable for use with gas chromatography and not requiring the use of toxic compounds. Wardle and Ghani (1995) also found SIR to be an effective method when used as a relative measure of glucose responsive (and metabolically active) microbial

biomass, particularly whenever the range of microbial biomass values is relatively small. SIR does have a few limitations, however. Because this method requires an incubation time of several hours, large numbers of samples are more difficult to handle (Schnürer and Rosswall, 1982). Also, it is assumed that in response to glucose, all the organisms release an equivalent amount of CO<sub>2</sub> per unit weight biomass, which may not be a correct assumption (Wardle and Ghani, 1995).

A more direct way of measuring soil microbial biomass is through direct microscopy. In this technique, the microorganisms are differentially stained. They can then be viewed under various optical microscopic methods and be counted directly (Anderson and Domsch, 1978). Microscopic techniques that have been used include: classical staining techniques, fluorescent staining, immunofluorescence, stereoscan electron microscopy, autoradiography, and infrared film photography (Babiuk and Paul, 1970). Babiuk and Paul (1970) found that fluorescent staining, specifically fluorescein isothiocyanate (FITC), was useful in that it allowed the fluorescent organisms to be differentiated from non-fluorescent clay particles. They also found that while direct microscopy was useful for estimating microbial biomass, plate counting provided a good estimate of metabolizing cells in soil. There are a number of limitations to the direct microscopy method. It requires skilled staff to do the counts, and even then it may be difficult to differentiate between living and dead cells. Also, many assumptions are made when the counts are converted to weights that may not be correct (Anderson and Domsch, 1978). This method may not be ideal for assessing large numbers of samples due to the time constraint.

Another way of determining soil microbial biomass utilizes ATP concentrations. ATP measurements have been used to obtain measures of biomass activity similar to those obtained by other procedures; however, the ATP content is influenced by P concentrations of the soil and other amendments, which limits its use for soil incubation experiments (Smith et al., 1985; Smith and Paul, 1990). This method provides only biomass C values

and must be calibrated through chloroform fumigation or direct microscopy (Smith and Paul, 1990). One advantage of this method is that no incubation period is necessary because the analysis is made on ATP extracted from cells (Schnürer and Rosswall, 1982). Jenkinson and Oades (1979) found the method to be very sensitive, although it requires careful extraction to prevent hydrolysis of ATP, and special equipment is necessary. Hulm et al. (1991) reported low reproducibility of the ATP results, and stated that the method was fairly tedious for routine testing.

Ninhydrin-reactive nitrogen (NRN) analysis is similar to the extraction procedure of the chloroform fumigation (FE) method except that it assays for NRN instead of C (Hulm et al., 1991). This method can give values for biomass C and N, as both elements can be correlated to the measurements of NRN (Amato and Ladd, 1988). Amato and Ladd (1988) found this method to be a sensitive assay of biomass C and N. However, Hulm et al. (1991) found the NRN technique underestimated biomass (compared to SIR) and had poor reproducibility, although the technique was reportedly simple to use.

Dehydrogenase activity has also been used to measure the microbial community, usually as a general measure of soil microbial activity (Hulm et al., 1991). The enzyme dehydrogenase is active inside intact and living cells (Brookes, 1995). The test for its activity is commonly used, but not very sensitive. When the microbial activity is low, several hours of incubation time may be required (Schnürer and Rosswall, 1982). Dehydrogenase activity may be a valuable indicator for some heavy metals, but Chander and Brookes (1991a) found that Cu tends to interfere with the assay measurement.

Fluorescein diacetate (FDA) hydrolysis may also be used to measure microbial biomass, though it may be better used to determine total heterotrophic activity (Schnürer and Rosswall, 1982). This technique allows for the determination of amounts of active fungi and bacteria, and can also locate acetylsterases in living protist cells (Schnürer and Rosswall, 1982). It has been suggested that this method could be used to determine the microbial biomass in many habitats (Swisher and Carroll, 1980). The advantages of this

method are that it is simple, rapid, sensitive, good for large sample numbers, and should be useful in comparative studies of microbial activity in natural habitats (Schnürer and Rosswall, 1982). There have been reported problems with this method at high and low pHs, but FDA hydrolysis shows promise as a general indicator of microbial activity (Schnürer and Rosswall, 1982).

Although soil microbial biomass measurements are commonly used to assess the microbial activity of soil, Wardle and Ghani (1995) point out that these measurements perhaps may not be the best bioindicator in all cases, and that there are other measurements based on biomass data that may be better. They mention the metabolic quotient (respiration : biomass ratio) (Anderson and Domsch, 1985) and the ratio of microbial C-to-organic C (Insam and Domsch, 1988) as two measurements that are strongly indicative of soil quality and are often more sensitive to ecological changes than microbial biomass by itself. There also has been some concern over how accurate the calibration equations used to estimate microbial biomass are when applied to some sampling conditions (Wardle and Ghani, 1995). However, this has not been found to cause excessive problems when the equations are used properly. Despite these possible limitations, microbial biomass measurements are routinely used and are relatively good indicators of the environmental health of soil.

**CASE STUDY:**  
**J-Field Ecological Risk Assessment Phase I Report**  
**Hlohowskyj et al., 1995**

This study was conducted by the EAD of Argonne National Laboratory in 1995. In this experiment, substrate-induced respiration was used to determine how metal contamination affected microbial activity in soil. For the toxic burning pit site, it was found that there was a significant increase in total heavy metal concentrations in the pushout area (TBTF) of the toxic burning pit site when compared to both local background (TBTC) and reference sites. The pushout area contains the areas where most of the waste disposal occurred. The local background area is located in an area of the toxic burning pit site where little waste disposal occurred. The reference sites are from an area in Maryland that has similar environmental characteristics as the toxic burning pit site; this reference area did not experience waste disposal. The mean total concentrations ( $\pm$  S. E.) of 7 heavy metals (As, Cd, Cr, Cu, Ni, Pb, Zn) at the reference, local background (TBTC), and pushout (TBTF) sites were, respectively,  $1.75 \pm 0.09$ ,  $7.7 \pm 0.3$ , and  $39.5 \pm 3.2$  mM kg<sup>-1</sup> dry mass of soil. Therefore, total heavy metal concentration at the local background site was 4.4 times greater than the reference site; at the pushout area total heavy metal concentration was 22.5 times greater than at the reference site.

Soil parameters in the contaminated, local background, and reference sites of the TBP area are shown in Table 1. The heavy metal concentrations of the pushout area were significantly greater (except for Se) than the local background and reference sites. The pH of the pushout area was found to be more alkaline (8.16) than either the local background (6.14) or the reference site (5.54). The percent organic matter was significantly higher in the reference site than in either the pushout or local background areas. This result was not anticipated, as this area was expected to have increased microbial activity which would lead to a decrease in organic matter. However, the microbial community of the reference

sites were frequently disturbed by cultivation, thereby causing the increase in organic matter due to decreased mineralization levels.

Spatial distribution of total concentrations of Cu, Pb, and Zn are shown in Figure 2. This distribution clearly illustrates the increasing heavy metal concentrations in the pushout area compared to the local background site.

This study found that microbial activity as measured by the SIR method was reduced in soils from the pushout area. This decrease in activity was paralleled by a decrease in the microbial biomass (Table 2). Table 2 shows the values for three soil microbial parameters for the reference site and both TBP sites. There was a significant difference in SIR rate between the reference site and the contaminated sites. The difference in the SIR rate between the two contaminated sites was not significant. However, the pushout site (TBTF) had considerably lower cumulative respiration than the local background (TBTC) (Figure 3).

There was also a significant difference between the reference site and the more contaminated TBTF site in active bacterial and total fungal biomass (Table 2). Microbial biomass estimated by the direct count method was depressed in the local background (TBTC) and the pushout area (TBTF) of the TBP site relative to the reference site. Total fungal biomass for the local background and contaminated sites were 54% and 15% that of the reference site. Active bacterial biomass was 71% and 19% that of the reference site (Table 2). However, the difference in active bacterial biomass levels between the pushout area and the local background site was not significant, although the difference in total fungal biomass was significant (Table 2).

Activity of carbon-, nitrogen- and phosphorus-acquiring enzymes was also significantly lower in the local background (TBTC) and contaminated sites (TBTF) compared with the reference site (Table 3). Significant reduction in the activities of all enzymes closely paralleled the increase in heavy metal concentrations. Ten-to-fifty fold reductions in enzyme activities were observed as heavy metal concentrations increased

(Figures 4-6). Relative to the reference site, the local background (TBTC) and contaminated (TBTF) sites exhibited enzyme activities that were only 14%-24% and 2%-10% for carbon- and nitrogen acquiring enzymes, and 23%-40% and 5%-49% for phosphorus-acquiring enzymes, respectively (Figures 7 and 8). This study also found that all enzyme activities and total fungal biomass were significantly correlated for all samples, providing greater confidence in the reliability of both enzyme and microbial data sets (Figures 9-11). These results suggest that soil contamination may have detrimental effects on the rates of organic matter degradation and subsequent release of nutrients to aboveground communities in the area.

The data indicate that the response of microbial communities to heavy metal contamination involves a threshold effect. There were no significant differences in the microbial activity, active biomass, or enzyme activity between the local background and the pushout sites, while there was a significant difference in heavy metal concentration (higher in the pushout area). Compared with these two sites, the reference site had much higher microbial activity, active biomass, and enzyme activity coupled with significantly lower heavy metal concentrations. This indicates that the main effect of heavy metals in the soil microbial community occurs at relatively low heavy metal concentrations.

Taken together, the results of this study showed the detrimental effects of heavy metal contamination on the soil microbial community. The toxic burning pit site of J-Field is now ready for a Phase 2 investigation which will further elucidate the ecological problems in that area. This investigation will include definitive toxicity testing to determine how organisms are affected by contaminated environmental media, additional residue analyses, and development of pathway models for estimating dose to higher trophic level receptors.



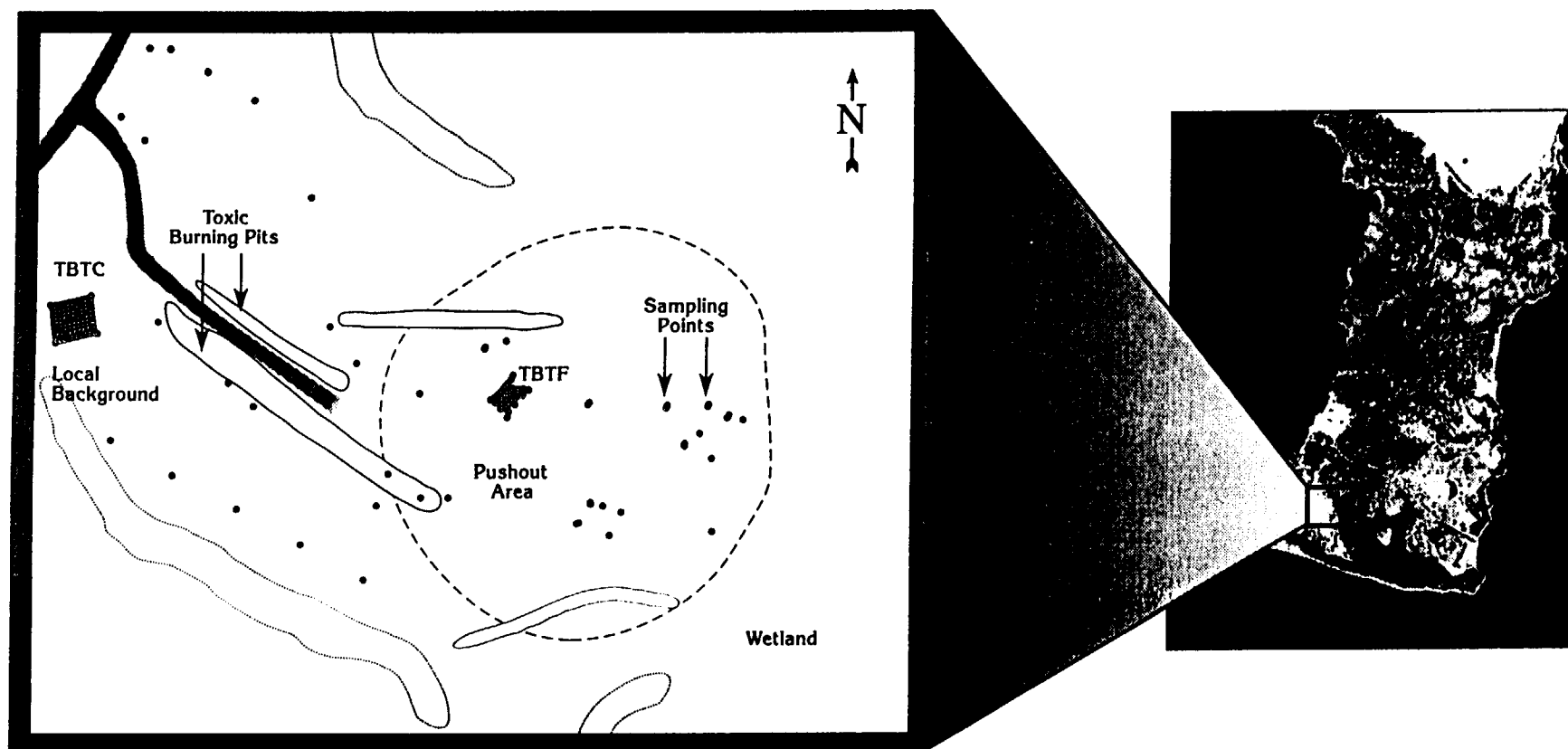


Figure 1. Location of survey grids in toxic burning pit areas of J-Field, Aberdeen Proving Ground, Gunpowder Neck Peninsula, Maryland.

Source: Hlohowskyj et al., 1995

**Table 1.** Soil parameters of the TBP site. Numbers presented as means. Statistical analyses were done on natural log transformed data.

Soil Parameter	Local Background (TBTC)	Pushout Area (TBTF)	Reference Site (RSA)	P-Value
As ( $\mu\text{g g}^{-1}$ )	6.10 <sup>ns</sup>	13.49	5.02 <sup>ns</sup>	0.0001
Cd ( $\mu\text{g g}^{-1}$ )	0.43 <sup>ns</sup>	3.90	0.20 <sup>ns</sup>	0.0001
Cr ( $\mu\text{g g}^{-1}$ )	42.31	122.87	23.60	0.0001
Cu ( $\mu\text{g g}^{-1}$ )	125.41	684.48	10.40	0.0001
Pb ( $\mu\text{g g}^{-1}$ )	250.21	2168.67	18.10	0.0001
Ni ( $\mu\text{g g}^{-1}$ )	12.59 <sup>ns</sup>	26.92	11.20 <sup>ns</sup>	0.0001
Zn ( $\mu\text{g g}^{-1}$ )	235.10	1787.97	51.50	0.0001
Se ( $\mu\text{g g}^{-1}$ )	0.14 <sup>ns</sup>	0.15 <sup>ns</sup>	0.168 <sup>ns</sup>	0.3276
Ca (lbs a <sup>-1</sup> )	897.69	1357.18	1234.0	0.0001
K (lbs a <sup>-1</sup> )	74.41	96.77	118.80	0.0001
Mg (lbs a <sup>-1</sup> )	171.97	1382.64	228.90	0.0001
CEC (meq)	3.03	9.44	6.90	0.0001
P (lbs a <sup>-1</sup> )	14.67	117.13	22.0	0.0001
O.M. (%)	1.43	1.68	4.25	0.0001
pH	6.14	8.16	5.54	0.0001

Note: Numbers with <sup>ns</sup> are not significantly different at  $\alpha=.05$  (Bonferroni mean separation test).

CEC = cation exchange capacity

O.M. = organic matter

Source: Hlohowskyj et al., 1995

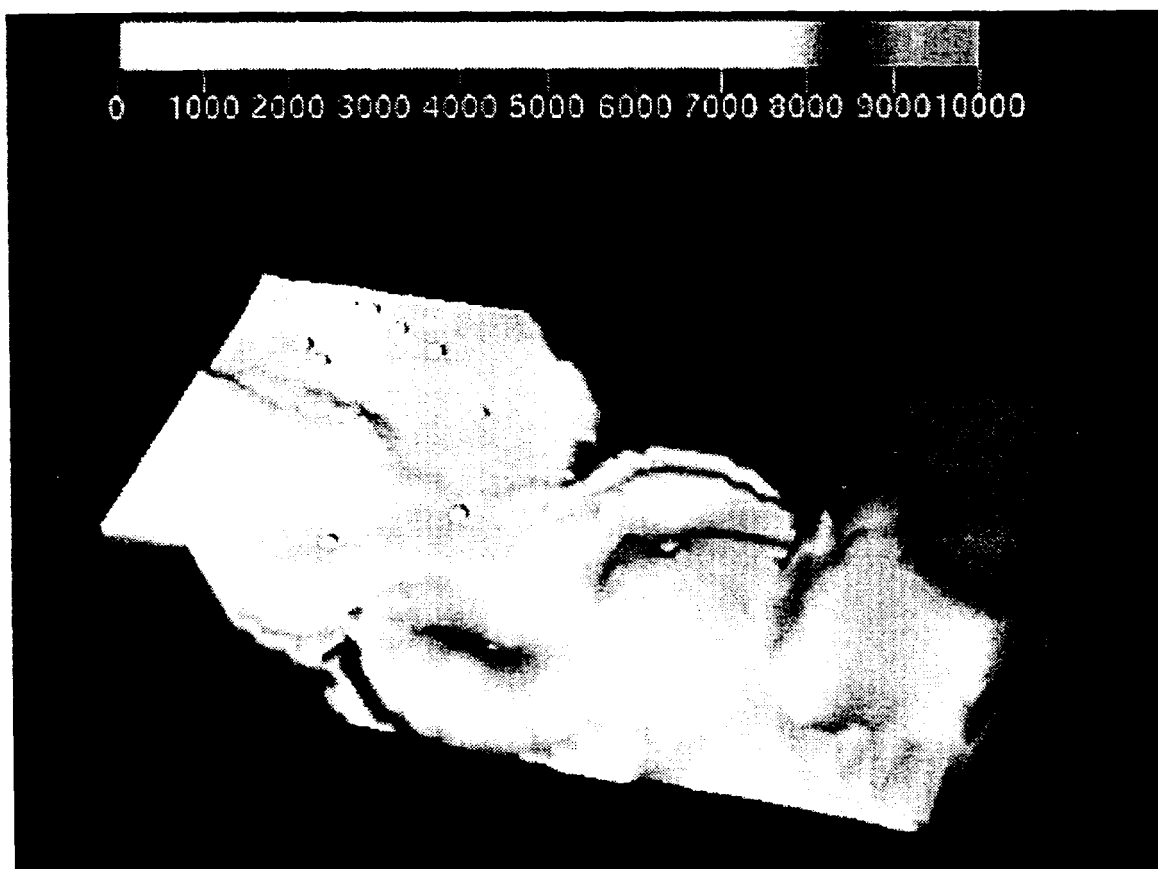


Figure 2. Spatial distribution of total concentrations of Cu, Pb, and Zn. The TBTC (local background) area is toward the left; the TBTF (pushout) area is toward the right.

Source: Hlohowskyj et al., 1995

**Table 2.** Microbial characteristics of two toxic burning pit sites containing heavy metals (TBTC = local background; TBTF = pushout area) and a reference site.

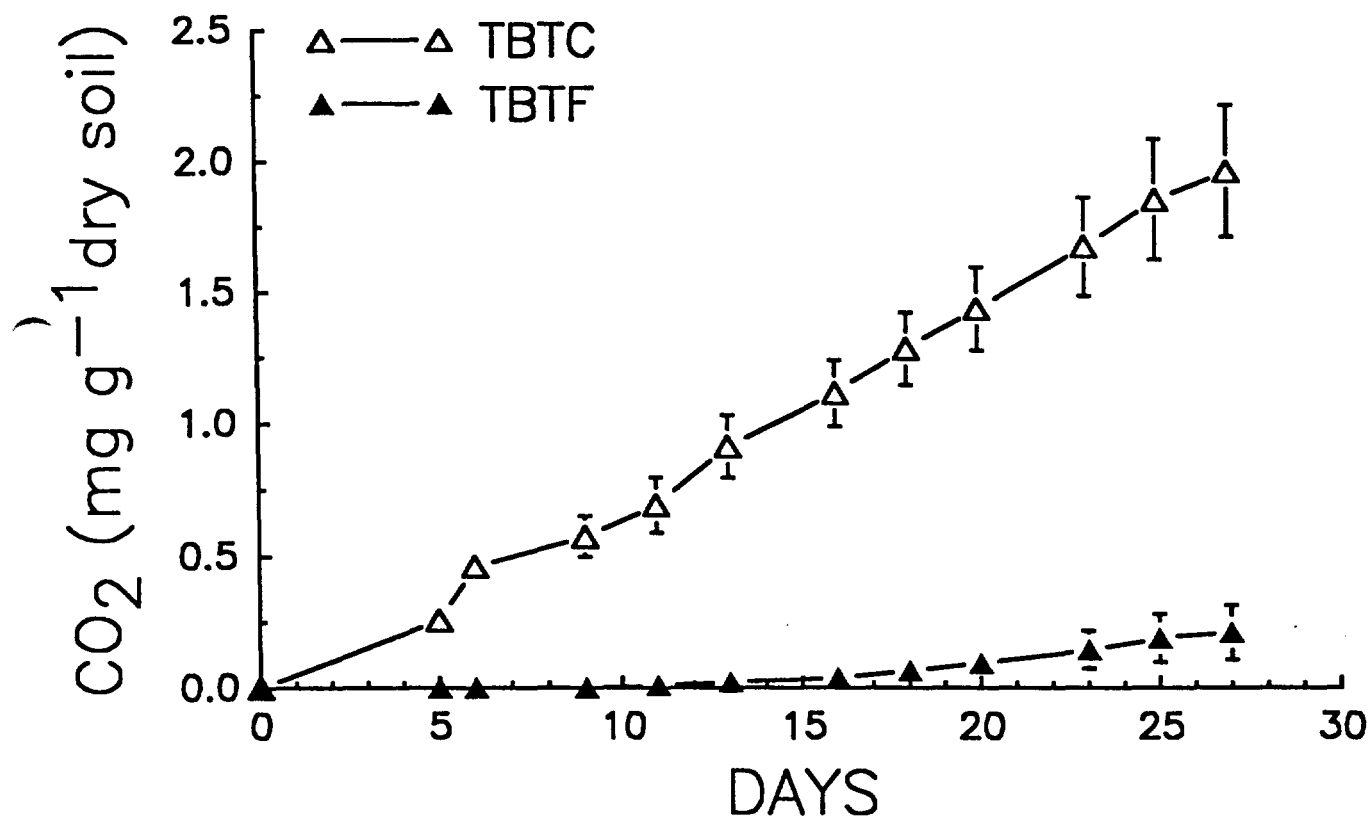
Soil microbial parameter	Reference site	TBTC	TBTF
Active bacterial biomass ( $\mu\text{g g}^{-1}$ DM)	15.37 <sup>a</sup>	10.94 <sup>ab</sup>	2.89 <sup>b</sup>
Total fungal biomass ( $\mu\text{g g}^{-1}$ DM)	335.27 <sup>a</sup>	182.95 <sup>a</sup>	48.96 <sup>b</sup>
SIR ( $\mu\text{g CO}_2 \text{ min}^{-1} \text{ g}^{-1}$ soil DM)	1.88 <sup>a</sup>	0.595 <sup>b</sup>	0.424 <sup>b</sup>

Note: Numbers with the same letter are not significantly different at  $\alpha=.05$ .  
DM = dry mass

Source: Hlohowskyj et al., 1995

Figure 3. Cumulative CO<sub>2</sub> evolution (mean  $\pm$  S. E.) in microcosms with soil from the terrestrial survey grids in the local background (TBTC) and the pushout areas (TBTF) of the toxic burning pit site.

### CUMULATIVE SOIL RESPIRATION



Source: Hlohowskyj et al., 1995

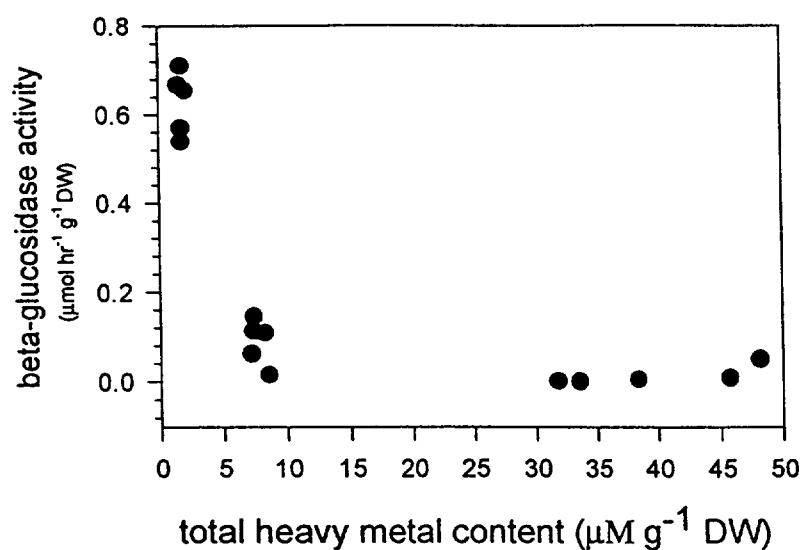
**Table 3.** Soil enzyme activities of two toxic burning pit sites containing heavy metals (TBTC = local background; TBTF = pushout area) and a reference site.

Soil enzyme	Reference site	TBTC	TBTF
Beta-glucosidase ( $\mu\text{M g}^{-1} \text{DM h}^{-1}$ )	0.63 <sup>a</sup>	0.10 <sup>b</sup>	0.01 <sup>c</sup>
Carboxymethyl cellulose (viscometric units $\text{g}^{-1} \text{DM h}^{-1}$ )	87.40 <sup>a</sup>	16.30 <sup>b</sup>	8.40 <sup>b</sup>
N-acetylglucosaminidase ( $\mu\text{M g}^{-1} \text{DM h}^{-1}$ )	0.27 <sup>a</sup>	0.07 <sup>b</sup>	0.02 <sup>c</sup>
Acid phosphatase ( $\mu\text{M g}^{-1} \text{DM h}^{-1}$ )	1.78 <sup>a</sup>	0.40 <sup>b</sup>	0.08 <sup>c</sup>
Alkaline phosphatase ( $\mu\text{M g}^{-1} \text{DM h}^{-1}$ )	0.30 <sup>a</sup>	0.12 <sup>b</sup>	0.15 <sup>b</sup>
Total phosphatase ( $\mu\text{M g}^{-1} \text{DM h}^{-1}$ )	2.08 <sup>a</sup>	0.52 <sup>b</sup>	0.23 <sup>c</sup>

Note: Numbers with the same letter are not significantly different at  $\alpha=.05$ .  
DM = dry mass

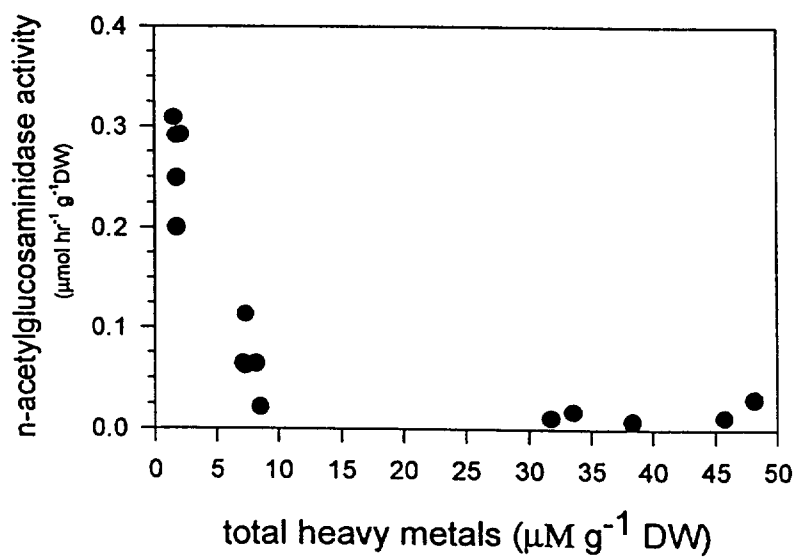
Source: Hlohowskyj et al., 1995

Figure 4. Effect of total heavy metal concentrations on carbon-acquiring enzyme activity in the soil of the toxic burning pit site.



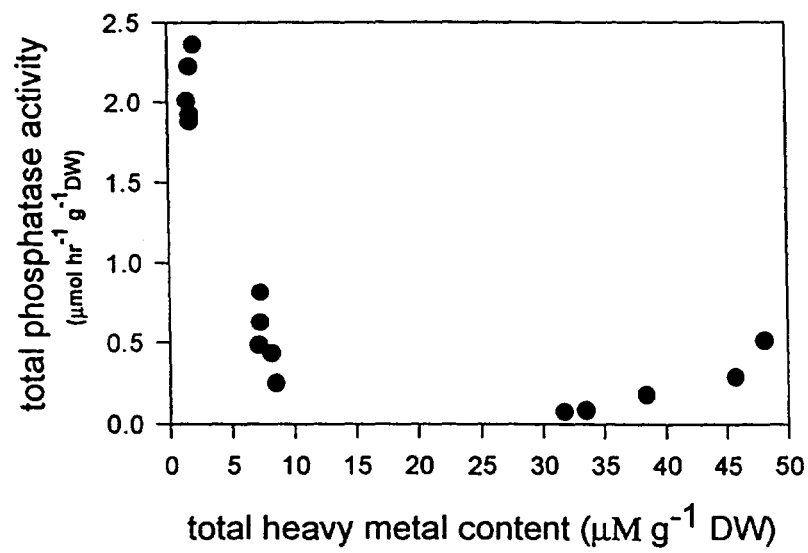
Source: Hlohowskyj et al., 1995

Figure 5. Effect of total heavy metal concentrations on nitrogen-acquiring enzyme activity in the soil of the toxic burning pit site.



Source: Hlohowskyj et al., 1995

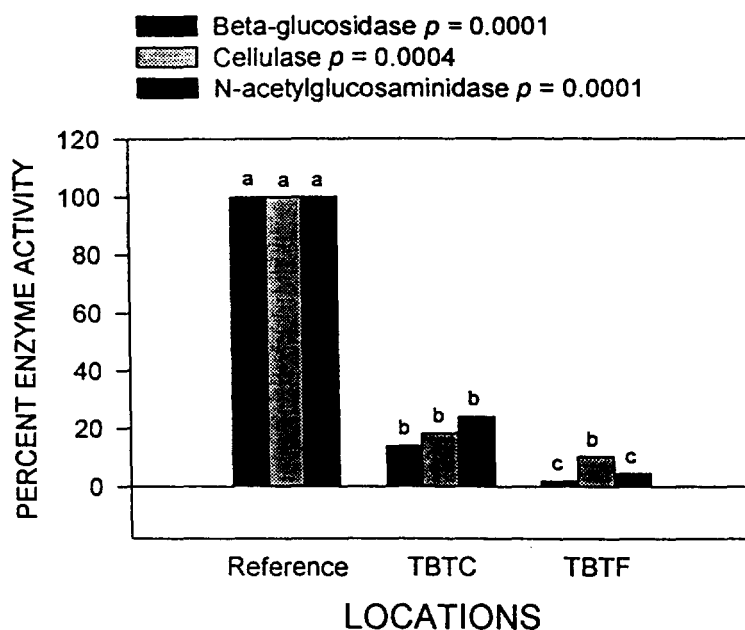
Figure 6. Effect of total heavy metal concentrations on phosphorus-acquiring enzyme activity in the soil of the toxic burning pit site.



Source: Hlohowskyj et al., 1995

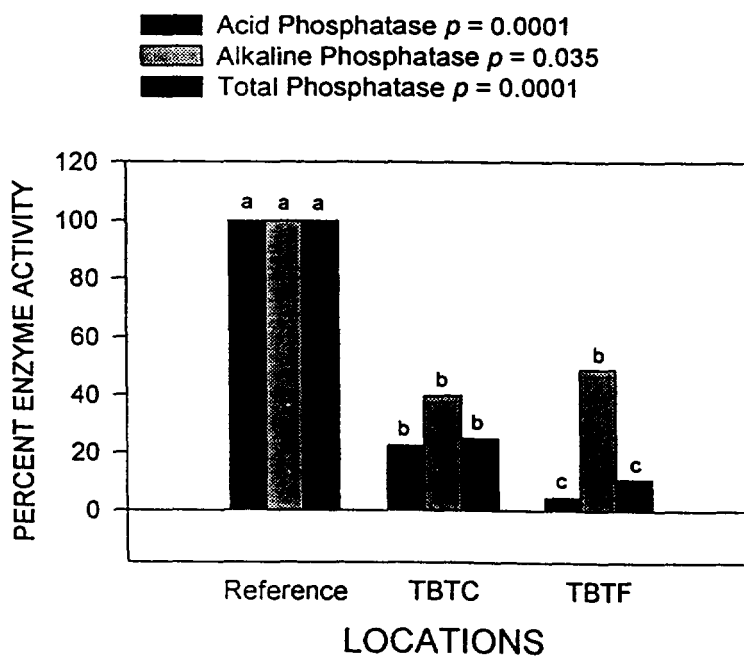


Figure 7. Changes in carbon- and nitrogen-acquiring enzyme activity in the soil of the toxic burning pit site.



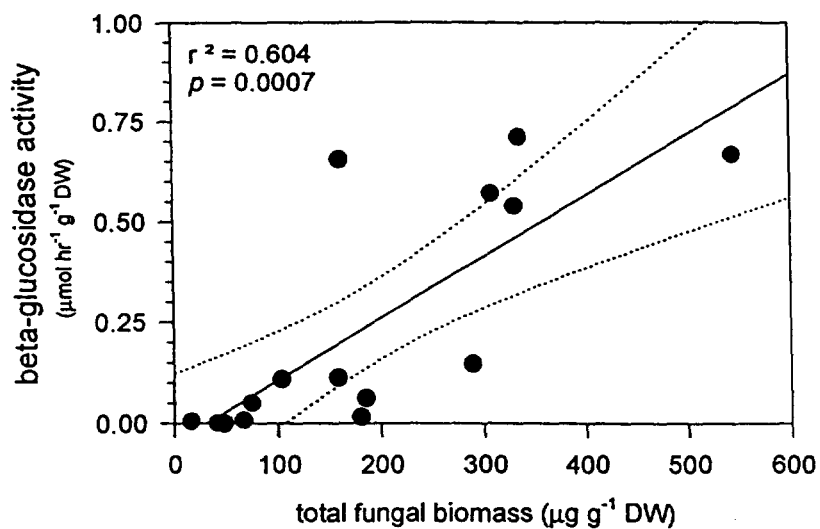
Source: Hlohowskyj et al., 1995

Figure 8. Changes in phosphorus-acquiring enzyme activity in the soil of the toxic burning pit site.



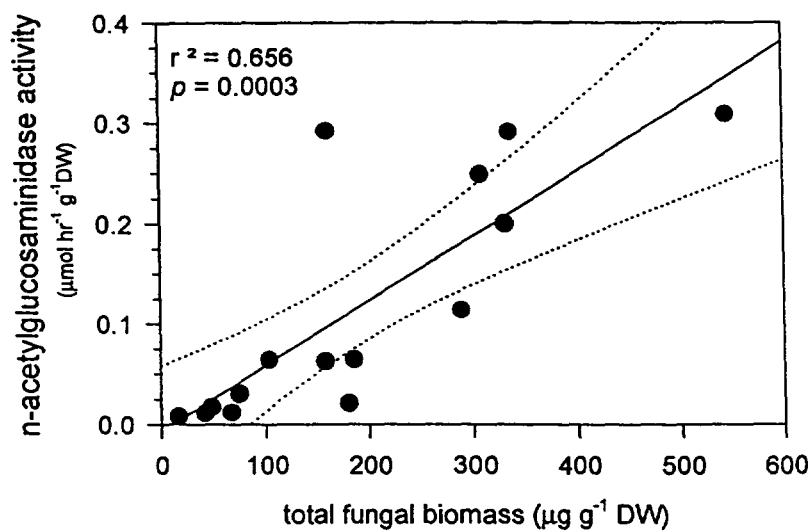
Source: Hlohowskyj et al., 1995

Figure 9. Relationship between carbon-acquiring enzyme activity and total fungal biomass in the soil of the toxic burning pit site (dotted lines show 95% confidence limits).



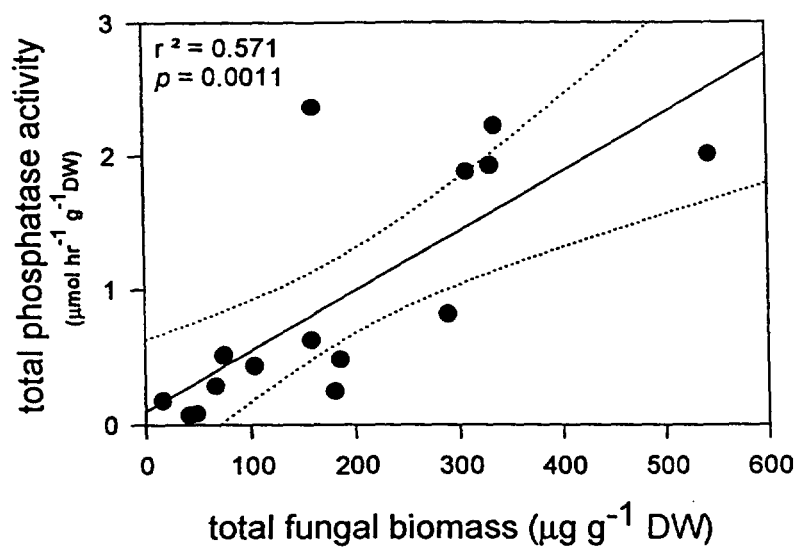
Source: Hlohowskyj et al., 1995

Figure 10. Relationship between nitrogen-acquiring enzyme activity and total fungal biomass in the soil of the toxic burning pit site (dotted lines show 95% confidence limits).



Source: Hlohowskyj et al., 1995

Figure 11. Relationship between phosphorus-acquiring enzyme activity and total fungal biomass in the soil of the toxic burning pit site (dotted lines show 95% confidence limits).



Source: Hlohowskyj et al., 1995

## REFERENCES

- Amato, M. and Ladd, J. N. 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biology & Biochemistry, 20(1): 107-114.
- Anderson, J. P. E. and Domsch, K. H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry, 10: 214-221.
- Anderson, T.-H. and Domsch, K. H. 1985. Determination of ecophysiological maintenance requirements of soil microorganisms in a dormant state. Biology and Fertility of Soils, 1: 81-89. As in Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Angle, J. S., Chaney, R. L., and Rhee, D. 1993. Bacterial resistance to heavy metals related to extractable and total metal concentrations in soil and media. Soil Biology & Biochemistry, 25(10): 1443-1446.
- Aoyama, M., Itaya, S., and Otowa, M. 1993. Effects of copper on the decomposition of plant residues, microbial biomass, and  $\beta$ -glucosidase activity in soils. Soil Science & Plant Nutrition, 39(3): 557-566.
- Bååth, E. 1992. Measurement of heavy metal tolerance of soil bacteria using thymidine incorporation into bacteria extracted after homogenization-centrifugation. Soil Biology & Biochemistry, 24(11): 1167-1172.
- Babiuk, L. A. and Paul, E. A. 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. Canadian Journal of Microbiology, 16: 57-62.
- Badia, D. and Alcañiz, J. M. 1993. Basal and specific microbial respiration in semiarid agricultural soils: Organic amendment and irrigation management effects. Geomicrobiology Journal, 11: 261-274.
- Bardgett, R. D. and Sagar, S. 1994. Effects of heavy metal contamination on the short-term decomposition of labelled [ $^{14}\text{C}$ ]glucose in a pasture soil. Soil Biology & Biochemistry, 26(6): 727-733.
- Barreiro, R. and Pratt, J. R. 1992. Toxic effects of chemicals on microorganisms. Water Environment Research, 64(4): 632-641.

- Brookes, P. C. 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils, 19: 269-279.
- Chander, K. and Brookes, P. C. 1991a. Is the dehydrogenase assay invalid as a method to estimate microbial activity in Cu-contaminated soils. Soil Biology & Biochemistry, 23: 901-1015. *As in* Brookes, P. C. 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. Biology and Fertility of Soils, 19: 269-279.
- Chander, K. and Brookes, P. C. 1991b. Microbial biomass dynamics during the decomposition of glucose and maize in metal-contaminated and non-contaminated soils. Soil Biology & Biochemistry, 23: 917-925. *As in* Fließbach, A., Martens, R., and Reber, H. H. 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biology & Biochemistry, 26(9): 1201-1205.
- Cheng, W. and Virginia, R. A. 1993. Measurement of microbial biomass in Arctic tundra soils using fumigation-extraction and substrate-induced respiration procedures. Soil Biology & Biochemistry, 25(1): 135-141.
- Coleman, D. C. 1985. Through a ped darkly: An ecological assessment of root-soil-microbial-faunal interactions. *In* Fitter, A. H., Atkinson, D., Read, D. J., and Usher, M. B. (eds.), Ecological Interactions in Soil, Blackwell Scientific Publications, Cambridge, U. K., p. 1-21.
- Dick, W. A. 1991. Introduction to Soil Biochemistry. Spring Quarter Agronomy 760 at the Ohio State University.
- Dindal, D. 1990. Soil Biology Guide, John Wiley and Sons, p. 1349.
- Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.
- Fließbach, A., Martens, R., and Reber, H. H. 1994. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biology & Biochemistry, 26(9): 1201-1205.
- Freedman, B. and Hutchinson, T. C. 1980. Effects of smelter pollutants on forest leaf litter decomposition near a nickel-copper smelter at Sudbury, Ontario. Canadian Journal of Botany, 58: 1722-1736. *As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.

- Harden, T., Joergensen, R. G., Meyer, B., and Wolters, V. 1993. Soil microbial biomass estimated by fumigation-extraction and substrate-induced respiration in two pesticide-treated soils. Soil Biology & Biochemistry, 25(6): 679-683.
- Hendricks, C. W. and Pascoe, N. 1988. Soil microbial biomass estimates using 2450 MHz microwave irradiation. Plant and Soil, 110: 39-47.
- Hendrix, P. F., Parmelee, R. W., Crossley, D. A., Jr., Coleman, D. C., Odum, E. P., and Groffman, P. M. 1986. Detritus foodwebs in conventional and no-tillage agroecosystems. Bioscience, 36: 374-380.
- Hlohowskyj, I., Hayse, J., Kuperman, R., and VanLonkhuyzen, R. 1995. J-Field Ecological Risk Assessment Phase I Report, Argonne National Laboratory.
- Hulm, S. C., Castle, D. L., Cook, K., Self, A., and Wood, M. 1991. Evaluation of soil microbial biomass methodology. Toxicological and Environmental Chemistry, 30: 183-192.
- Ingham, E. R. and Coleman, D. C. 1984. Effects of streptomycin, cycloheximide, fungizone, captan, carbofuran, cygon, and PCNB on soil microbe populations and nutrient cycling. Microbial Ecology, 10: 345-358.
- Ingham, E. R., Trofymow, J. A., Ames, R. N., Hunt, H. W., Morley, C. R., Moore, J. C., and Coleman, D. C. 1986a. Trophic interactions and nitrogen cycling in a semiarid grassland soil. Part I. Seasonal dynamics of the soil foodweb. Journal of Applied Ecology, 23: 608-615.
- Ingham, E. R., Trofymow, J. A., Ames, R. N., Hunt, H. W., Morley, C. R., Moore, J. C., and Coleman, D. C. 1986b. Trophic interactions and nitrogen cycling in a semiarid grassland soil. Part II. System responses to removal of different groups of soil microbes or fauna. Journal of Applied Ecology, 23: 615-630.
- Ingham, E. R. and Horton, K. A. 1987. Bacterial, fungal, and protozoan responses to chloroform fumigation in stored prairie soil. Soil Biology & Biochemistry, 19: 545-550.
- Insam, H. and Domsch, K. H. 1988. Relationship between soil organic C and microbial biomass on chronosequences of reclamation sites. Microbial Ecology, 15: 177-188. As in Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Jenkinson, D. S. 1987. Determination of microbial biomass carbon and nitrogen in soil. In Wilson, J. R. (ed.), Advances in Nitrogen Cycling in Agricultural

- Ecosystems, p. 368-386, CAB International, Wallingford. *As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.
- Jenkinson, D. S. and Ladd, J. N. 1981. Microbial biomass in soils: measurement and turnover. *In* Paul, E. A. and Ladd, J. N. (eds.). Soil Biochemistry, 5: 415-471, Dekker, New York. *As in* Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Jenkinson, D. S. and Powlson, D. S. 1976. The effect of biocidal treatment on metabolism in soil. V. A method for measuring soil biomass. Soil Biology & Biochemistry, 8: 209-213. *As in* Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Joergensen, R. G. and Brookes, P. C. 1991. Soil microbial biomass estimations by fumigation extraction. Mitteilungen der Deutschen Bodenkundlichen Gesellschaft, 66: 511-514. *As in* Harden, T., Joergensen, R. G., Meyer, B., and Wolters, V. 1993. Soil microbial biomass estimated by fumigation-extraction and substrate-induced respiration in two pesticide-treated soils. Soil Biology & Biochemistry, 25(6): 679-683.
- Jordan, M. J. and Lechevalier, M. P. 1975. Effects of zinc-smelter emissions on forest soil microflora. Canadian Journal of Microbiology, 21: 1855-1865. *As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.
- Lepp, N. W. 1992. Uptake and accumulation of metals in bacteria and fungi. *In* Adriana, D. C. (ed.), Biogeochemistry of Trace Metals, Lewis Publishers, Boca Roton, p. 277-298.
- Martino, L., Ditmars, J. D., and Stachiw, K. P. 1995. Work Plan for Conducting an Ecological Risk Assessment at J-Field, Aberdeen Proving Ground, Maryland, Argonne National Laboratory.
- Mathur, S. P. 1981. The inhibitory role of copper in the enzymatic degradation of organic soils. Proceedings of the International Peat Symposium, Bemidji, Minnesota (USA). *As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.

- McGrath, S. P. 1994. Effects of heavy metals from sewage sludge on soil microbes in agricultural ecosystems. *In* Ross, S. M. (ed.), Toxic Metals in Soil-Plant Systems, John Wiley & Sons Ltd, p. 247-274.
- Mueller T., Joergensen, R. G., and Meyer, B. 1992. Estimation of soil microbial biomass C in the presence of fresh roots by fumigation-extraction. Soil Biology & Biochemistry, 24: 179-181. *As in* Harden, T., Joergensen, R. G., Meyer, B., and Wolters, V. 1993. Soil microbial biomass estimated by fumigation-extraction and substrate-induced respiration in two pesticide-treated soils. Soil Biology & Biochemistry, 25(6): 679-683.
- Nannipieri, P., Grego, S., and Ceccanti, B. 1990. Ecological significance of the biological activity in soil. Soil Biochemistry, 6: 283-355.
- Németh, T., Molnár, E., Csillag, J., Bujtás, K., Lukács, A., Pártay, G., Fehér, J., and van Genuchten, M. Th. 1993. Mobility of some heavy metals in soil-plant systems studied on soil monoliths. Water Science & Technology, 28(3-5): 389-398.
- Ocio, J. A. and Brookes, P. C. 1990. An evaluation of methods for measuring the microbial biomass in soils following recent additions of wheat straw and the characterization of the biomass that develops. Soil Biology & Biochemistry, 22: 685-694. *As in* Harden, T., Joergensen, R. G., Meyer, B., and Wolters, V. 1993. Soil microbial biomass estimated by fumigation-extraction and substrate-induced respiration in two pesticide-treated soils. Soil Biology & Biochemistry, 25(6): 679-683.
- Okano, S., Kondo, H., and Sawada, Y. 1989. Soil microbial biomass in a sasa-type (*Pleioblastus*) grassland. Japanese Journal of Grassland Science, 34: 280-285. *As in* Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Ortiz, O. and Alcañiz, J. M. 1994. Respiration potential of microbial biomass in a calcareous soil treated with sewage sludge. Geomicrobiology Journal, 11: 333-340.
- Reber, H. H. 1992. Simultaneous estimates of the diversity and the degradative capability of heavy-metal-affected soil bacterial communities. Biology and Fertility of Soils, 13: 181-186.
- Rühling, Å. and Tyler, G. 1973. Oikos, 24: 402. *As in* Tyler, G., Balsberg Pålsson, A.-M., Bengtsson, G., Bååth, E., and Tranvik, L. 1989. Heavy-metal ecology of terrestrial plants, microorganisms and invertebrates. Water, Air, and Soil Pollution, 47: 189-215.



- Santrukova, H. 1992. Zdroje chyb pri stanoveni mikrobni biomasy fumigacni metodou. Rostlinna Vyroba, 38: 893-898. *As in* Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Schnürer, J. and Rosswall, T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied and Environmental Microbiology, 43(6): 1256-1261.
- Singh, J. S., Raghubanshi, A. S., Singh, R. S., and Srivastava, S. C. 1989. Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. Nature, 338: 499-500. *As in* Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Smith, J. L., McNeal, B. L., and Cheng, H. H. 1985. Estimation of soil microbial biomass: An analysis of the respiratory response of soils. Soil Biology & Biochemistry, 17(1): 11-16.
- Smith, J. L. and Paul, E. A. 1990. The significance of soil microbial biomass estimations. *In* Bollag, J.-M. and Stotzky, G. (eds.), Soil Biochemistry, 6: 357-397, Marcel Dekker, Inc., New York.
- Starzecka, A. and Bednarz, T. 1993. Comparison of development and metabolic activity of algae and bacteria in soils under the influence of short- and long-term contamination with metallurgic industrial dusts. Algological Studies, 70: 71-88.
- Swisher, R. and Carroll, G. C. 1980. Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces. Microbial Ecology, 6: 217-226. *As in* Schnürer, J. and Rosswall, T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied and Environmental Microbiology, 43(6): 1256-1261.
- Tyler, G. 1974. Heavy metal pollution and soil enzymatic activity. Plant and Soil, 41: 303-311. *As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.
- Tyler, G. 1975. Heavy metal pollution and mineralization of nitrogen in forest soils. Nature, Lond., 255: 701-702. *As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in

metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.

- Tyler, G. 1976. Heavy metal pollution, phosphatase activity and mineralization of organic phosphorus in forest soils. Soil Biology & Biochemistry, 8: 327-332.
- As in* Dumontet, S. and Mathur, S. P. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biology & Biochemistry, 21(3): 431-436.
- Tyler, G., Balsberg Pålsson, A.-M., Bengtsson, G., Bååth, E., and Tranvik, L. 1989. Heavy-metal ecology of terrestrial plants, microorganisms and invertebrates. Water, Air, and Soil Pollution, 47: 189-215.
- Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Wardle, D. A. and Parkinson, D. 1990. Comparison of physiological techniques for estimating the response of the soil microbial biomass to soil moisture. Soil Biology & Biochemistry, 22: 817-823. *As in* Wardle, D. A. and Ghani, A. 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry, 27(6): 821-828.
- Yeates, G. W., Orchard, V. A., Speir, T. W., Hunt, J. L., and Hermans, M. C. C. 1994. Impact of pasture contamination by copper, chromium, arsenic timber preservative on soil biological activity. Biology and Fertility of Soils, 18: 200-208.

